

Polymerase Chain Reaction

Overview :

This protocol is generously provided by the lab.

Materials :

Component	20µL reaction	50µL reaction	Final cocentration
ddH2O	To 20µL	To 50 µL	
5X Phusion GC Buffer	4 µL	10 µL	1X
10 mM dNTPs	0,4 µL	1 µL	200 µM
10 µM Forward Primer	1 µL	2,5 µL	0,5 µM
10 µM Reverse Primer	1 µL	2,5 µL	0,5 µM
PhUsion DNAPol	0,2 µL	0,5 µL	1.0 units/50µL PCR
Template DNA	- Use 1pg–1ng of plasmid templates - Use 1ng–1µg of genomic templates		

Program :

Step	Temperature	Time	Cycles
Initial denaturation	94°C	5 min	x 1
Denaturation	94°C	30 sec	x 35
Annealing	X°C	30 sec	
Elongation	72°C	Y:YY	
Final extention	72°C	10 min	x 1
Final hold	15°C	∞	x 1

X°C – Annealing temperature - 5°C

Y:YY – Extension time (1 min/kb)